46. Takeo Tsukamoto and Tetsuya Komori: Studies on Visual Function. II.[1] Allied Compounds of Arecoline.

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During the course of investigations on the relative action of various toxic substances and vitamins, one of the authors (Tsukamoto), in cooperation with Hosoya and Ogoh, found that arecoline, the effective component of the Areca nut, possessed marked actions in the regeneration of visual purple and acceleration of visual acuity in the dark. Moreover, such actions were found to be increased by the concurrent presence of vitamin A and C.[2] The effect of some newly synthesized compounds related to arecoline on the system of visual sensation was examined in order to find the changes in the increased activity of visual acuity by the administration of various chemicals and their action mechanism.

The chemicals used for such examinations were newly synthesized.[3] Pharmacological action of such compounds was examined by the observation of regeneration faculty of visual purple in the retina,[4] for the changes in the receptor alone, and the details are reported in the present and the following papers. At the same time, transmission process of excitement was observed in cooperation with the Physiology Department. Relationship with the potential of retina activity will be reported elsewhere.[5]

There are many reports on the paper partition chromatography of amino acids, and Lenshner[6] and Hashizume[7] reported on such procedures with pyridine-carboxylic acids but no such work seems to have been published on piperidine-carboxylic acids. As a means of separatory estimation, paper partition chromatography was carried out in the present series of experiments, with arecoline hydrobromide as the chief objective, and making comparative examination of various piperidine-carboxylic acids, the results of which are shown in Table I.

Table I. Rf Values of Arecoline and Some of Its Related Compounds

	Solvent	No. 1	No. 2	No. 3	No. 4.	No. 5	No. 6
	System (Ratio in volume)	BuOH 5 AcOH 1 H_2O 4	-do-	AmOH 5 AcOH 1 H_2O 4	BuOH 5 AcOEt 2 H_2O 3	BuOH 1 H_2O 1	AmOH 2 Py 1 H_2O 1
	Temp.(°C)	18~20	30~31	27~28	25~31	28~31	28~31
	Compd. Time(hrs.)	8	8	5~7	3~6	4	4
1	Arecaidine-HBr	0.42	0.35	0.12~0.13	0.05±0.01	0.05	0.03
2	Dihydroarecaidine-HBr	0.44	0.36	0.16~0.15	0.07±0.01	0.07±0.01	0.04
3	Arecoline-HBr	0.60	0.52	0.37~0.36	0.71~0.72	0.84±0.01	0.87~0.86
4	Arecoline-HCl	0.60	0.52	—	0.71	—	—
5	Arecaidine Et ester-HCl	0.73	0.70	0.49±0.01	0.83±0.01	0.89±0.01	0.91~0.90
6	Arecaidine Pr ester-HCl	0.79	0.76	0.61±0.01	0.93	top	top
7	Arecaidine isoPr ester-HCl	0.82	0.80	0.62±0.01	0.90	top	top
8	Arecaidine Bu ester-HCl	0.85	0.84	0.72±0.01	0.94~0.95	top	top
9	Et isonipecotinate-HI	0.73~0.74	0.68	0.47±0.01	0.73~0.74	0.69	0.81
10	N-Me-nipecotinamide-HI	0.45	0.36	0.11±0.01	0.20~0.17	0.16~0.20	0.26
11	Et 2-Me-1,4,5,6-tetrahydro-nicotinate	0.68	0.73	—	top	top	top

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1) Tsukamoto, Komori: Synthesis of Some Arecaidine Derivatives (J. Pharm. Soc. Japan, **73**, 779 (1953)) will be designated Part I of this series. 2) Unpublished.

3) Part of the paper read before the Kyushu Local Meeting of the Pharmaceutical Society of Japan, October 22, 1954.

4) Paper read before the 75th General Meeting of the Pharmaceutical Society of Japan, April 10, 1955.

5) Paper read before the General Meeting of the Japanese Medical Association, April 3, 1955.

6) F. Lenshner: Naturwissenschaften, **40**, 554(1954).

7) T. Hashizume: Nature, **173**, 645(1954).

Many workers have reported on the ultraviolet absorption spectra of the α,β-unsaturated bond in many compounds and Glickmann[8] and Albertson[9] reported on the spectra of the structure of β-amino derivatives of α,β-unsaturated lactones and esters, using nitrogen-containing carboxylic acids related to arecoline. The ultraviolet absorption spectra were also determined by the present authors, noting the fact that carbonyl group and the double bonds in the piperidine skeleton were conjugated in arecoline and, as anticipated, there was no absorption band in the ultraviolet region for dihydroarecaidine hydrobromide (in longer wave lengths above 203 mμ), while arecaidine hydrobromide and arecoline hydrobromide respectively showed absorption bands at 205 mμ (H_2O) (ε_{max} 12.4×10^3) and 215.5 mμ(MeOH) (ε_{max} 7.2×10^3). The shift of the band in arecoline to a longer wave length region is due to the introduction of a substituent(Fig. 1). The ultraviolet absorption spectra of compounds possessing pyridine skeleton, such as picolinic acid methylbetaine hydriodide, methyl nicotinate methiodide, and ethyl isonicotinate methiodide, were all found to have bands of high intensity in the shorter wave length region, at 224 mμ, and bands of low intensity in the longer wave lengths, at 273, 264, and 274 mμ. These clearly demonstrate the presence of a conjugation between the carbonyl and the pyridine ring and, as pointed out by Doub and Vandenbelt[10] with many of the benzene derivatives, are assumed to be the bands at 190~200 mμ and 240~260mμ shifted to a longer wave length region.

In Fig. 2, it is seen that the molecular extinction coefficient, ε, of the secondary band in (I) is extremely large, compared to those of (II) and (III). This is rather an interesting fact compared to the fact that the coefficient of 1,2-di(γ-pyridyl)ethane is larger than that of γ-picoline.[11]

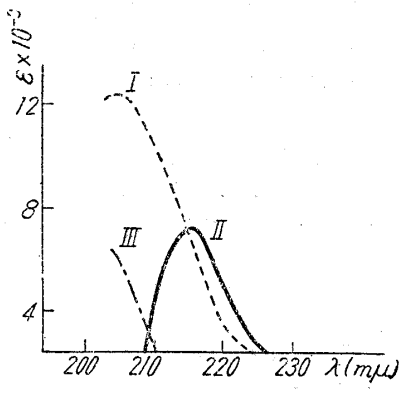

Fig. 1. Ultraviolet Absorption Spectra
I Arecaidine hydrobromide (H_2O)
II Arecoline hydrobromide (MeOH)
III Dihydroarecaidine hydrobromide (H_2O)

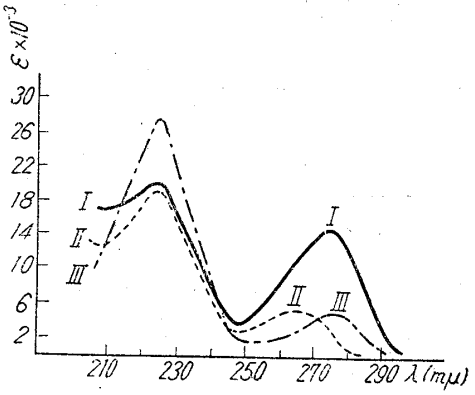

Fig. 2. Ultraviolet Absorption Spectra (in H_2O)
I Picolinic acid methylbetaine hydriodide
II Methyl nicotinate methiodide
III Ethyl isonicotinate methiodide

The decomposition point of arecaidine hydrobromide was given in the previous report[1] as 197° but in the present series of experiments, the decomposition point was raised to 247~249°,[12] through a hygroscopic barium salt of m.p. over 360°(decomp.).[13] These values agree well with the data given in the literature[14] and no melting point depression was

8) S. A. Glickmann, A. C. Cope: J. Am. Chem. Soc., **67**, 1017(1945).
9) N. F. Albertson: *Ibid.*, **74**, 3816(1952).
10) Doub, Vandenbelt: *Ibid.*, **69**, 2714(1947); **71**, 2114(1949).
11) M. Yamin, R. M. Fuoss: *Ibid.*, **75**, 4861(1953).
12) *Anal.* Calcd. for $C_7H_{11}O_2NBr\cdot\frac{1}{2}Ba$: C, 29.00; H, 3.80; N, 4.83. Found: C, 29.68; H, 4.053; N, 4.62.
13) *Anal.* Calcd. for $C_7H_{12}O_2NBr$: C, 37.85; H, 5.40; N, 6.309. Found: C, 37.72; H, 5.27; N, 6.61.
14) N. A. Preobrazhenskii, L. B. Fischer: J. Gen. Chem. (U. S. S. R.), **11**, 140(1941). cf. Hess, Leibbrandt: Ber., **51**, 813(1918); K. Freudenberg: *Ibid.*, **51**. 1668(1918).

observed on admixture with a sample obtained by the hydrolysis of arecoline hydrobromide with 4% hydrobromic acid. The compound with lower decomposition point gives identical ultraviolet absorption spectrum, Rf value, and the melting point of the picrate as those of the higher decomposition compound and it is assumed that the decomposition point had lowered due to occlusion of a minute amount of impurities.

The authors are indebted to Messrs. T. Hattori and T. Mayeda for the analytical data.

Experimental

Picolinic Acid Methylbetaine Hydriodide (I)—A mixture of 1.5 g. of methyl picolinate, 2.2 g. MeI, and 1.8 cc. abs. MeOH was sealed in a tube and heated at 100° for 1 hr. The crystals that separated out were removed by filtration, the filtrate was concentrated under a reduced pressure, and the crystals thereby obtained were combined with the earlier crystals, washed with ether, and recrystallized from MeOH to deliquescent yellow crystals, m.p. 160°(decomp.). *Anal.* Calcd. for $(C_7H_7O_2N)_2$·HI : C, 41.80; H, 3.73; N, 6.96. Found : C, 41.30; H, 3.48; N, 6.68. Ultraviolet absorption : $\lambda_{max}^{H_2O}$ 224 mμ (ε_{max} 20.35×10^3), 273 (14.74×10^3).

Methyl Nicotinate Methiodide (II)—Methyl nicotinate, MeI, and MeOH were treated as in the foregoing for (I) and yielded deliquescent yellow crystals of m.p. 124.5~126.5° *Anal.* Calcd. for $C_8H_{10}O_2NI$: C, 34.41; H, 3.58; N, 5.34. Found : C, 34.77; H, 3.76; N, 5.02. Ultraviolet absorption : $\lambda_{max}^{H_2O}$ 224 mμ (19.73×10^3), 264 (5.6×10^3).

Ethyl Isonicotinate Methiodide (III)—MeI was added to the EtOH solution of ethyl isonicotinate in slight excess of the calculated amount, the mixture was gently refluxed on a water bath, the crystals that separated out were collected by filtration, washed with ether, and recrystallized from EtOH–ether mixture to deliquescent yellow crystals, m.p. 122~124°. *Anal.* Calcd. for $C_9H_{12}O_2NI$: C, 36.80; H, 4.08; N, 4.78. Found : C, 36.77; H, 4.15; N, 4.99. Ultraviolet absorption : $\lambda_{max}^{H_2O}$ 224 mμ (27.84×10^3), 274 (5.57×10^3).

Ethyl N-Methylisonipecotinate Hydriodide (IV)—A solution of 0.5 g. of (III) dissolved in 95% EtOH, with 0.02 g. of PtO_2, was submitted to catalytic reduction at about 30°. After absorption of 3 moles of H_2, the catalyst was removed by filtration, the filtrate was concentrated under a reduced pressure, and the residual crystals were recrystallized from EtOH–ether mixture to white crystals, m.p. 125°. *Anal.* Calcd. for $C_9H_{18}O_2NI$: C, 36.12; H, 6.05; N, 4.68. Found : C, 36.04; H, 5.59; N, 4.58.

Arecoline Methiodide (V)—To a solution of arecoline hydrobromide dissolved in MeOH, a calculated amount of Ag_2O was added, shaken, and filtered. MeI was added to the filtrate, allowed to stand, and the precipitate obtained on the addition of ether was recrystallized from MeOH to crystals melting at 173°, agreeing with bibliographic data.[17]

Dihydroarecaidine Hydrobromide (VI)—To 1 part of arecaidine hydrobromide, m.p. 247~249° (decomp.), 0.1 part of PtO_2 and 5 parts of H_2O were added and the mixture was submitted to catalytic reduction at about 40° by which 1 mole of H_2 was absorbed quantitatively and afforded (VI) as white crystals, m.p. 196~198°.

To a solution of 1 g. of nicotinamide methiodide dissolved in about 5 cc. of water, 0.1 g. of PtO_2 was added and catalytically reduced at about 40°, resulting in the absorption of 3 moles of H_2. N-Methylnipecotinamide hydriodide, m.p. 178~180°, thereby obtained agreed with the bibliographical data.[18] The free base obtained on extraction of its KOH-alkaline solution with ether was added to about 50 volumes of 4% aq. solution of HBr, refluxed for 10 hrs., the solution was evaporated to dryness on a water bath under a reduced pressure, and the residue was recrystallized from MeOH–ether mixture after washing with EtOH. The product thereby obtained showed no depressin of m.p. on admixture with dihydroarecaidine hydrobromide. No absorption in the ultraviolet region in the longer wave lengths above 203 mμ. *Anal.* Calcd. for $C_7H_{14}O_2NBr$: C, 37.50; H, 6.25. Found: C, 37.51; H, 6.23.

Ethyl 2-Methyl-1,4,5,6-tetrahydronicotinate (VII)—Synthesized from ethyl acetoacetate and acrylonitrile according to the method of Albertson.[11] $b.p_{0.08}$ 115°. Shows absorption spectrum of ethyl β-aminocrotonate type, with absorption maximum at 289 mμ in EtOH. All agree with bibliographical data.

Paper Partition Chromatography—i) Apparatus and Methods : A glass cylinder of 6 cm. inside diameter and 40 cm. high, or 5 cm. in inside diameter and 30 cm. high, was used and by one-dimensional ascending method, strips of Toyo Roshi No. 2 of 2×5~10 cm. in width and 30 cm. in length

17) K. Freudenberg : Ber., **51**, 1668(1918).
18) K. Tomita : J. Pharm. Soc. Japan, **71**, 220(1951).

were submitted to the tests. On the line 3 cm. from the bottom, at 2-cm. intervals, 0.01 cc. each of 0.1 mg./cc. solution of the sample compound was spotted by a micropipet, and colored by the Dragendorff reagent, causing red spots to appear on a pale yellow background. When the spot was obscure, the strips were heated at 80° for 5 mins. and washed with water, by which the spots appeared clearly. The solutions were prepared in $CHCl_3$ for the compounds (4), (5), (6), (7), and (8), in water for (1), (2), and (10), in EtOH for (9) and (11), and in MeOH for (3), listed in Table I.

ii) Developing Medium : Upper layer or the respective mixtures was used, No. 1 after being allowed to stand for 3 days, No. 2 after 14 days, and No. 3 after 4 days.

Absorption Spectral Measurements—Shimadzu Spectrophotometer Type QB 50 was used. Usually, visible region from 203 mμ was measured. The solvents were purified by the usual method. Distilled water was used after two distillations. Quartz cell of 10-mm. optical depth was used. The intervals of measurement was ususlly 5~10 mμ or 0.5 mμ near the peaks.

Summary

In order to find out the changes in the acceleration of visual acuity in the dark by the administration of chemicals and their reaction mechanisms, compounds related to arecoline were synthesized.

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47. Takeo Tsukamoto and Tetsuya Komori : Studies on Visual Function. III.[1] Regeneration of Visual Purple by Allied Compounds of Arecoline.

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Decomposition of rhodopsin by photo-impulsion is regenerated immediately after interception of the excitement and the rate of this regeneration depends on various conditions and factors. Change of regeneration rate by the administration of chemicals has been observed by many and pilocarpine is said to accelerate the reaction while cocaine suppresses it. Examinations have been made as to the effect of scopolamine, acetylcholine, arecoline, methionine, betaine, physostigmine, cysteine, cystine, and creatinine, but no systematic studies have been carried out.

In the previous paper,[1] syntheses of some allied compounds of arecoline were described. Observations as to their action mechanism will be deferred to a later date and, as a fundamental experiment, comparative examination of these compounds on the rhodopsin regeneration of the retina, as the change of the receptor alone, will be described below.

Methods

Toads (*Bufo vulgaris* formosus *Boulenger*) of constant weight, with identical external appearance and size of eyes, were chosen as the experimental animals. After light adaptation in the light adaptation apparatus, 0.01 mole of each of the sample compound was injected into the abdominal lymph sac of the toad as 0.6 cc./200 g. Ringer solution for cold-blooded animals, and the animals were immediately submitted to dark adaptation. The concentration of rhodopsin in the retina after 30 and 60 mins. was compared with those of the control animal given only the Ringer solution and after dark adaptation.

Operation in the Light—The light-adaptation apparatus was a white enamelled tank of 24 cm. in inside diameter and 24 cm. in height. About 1 cm. above the tank, a glass plate was placed and about 3 cm. above this glass plate, Mazda incandescent lamp of 300 W, 100 V., was placed, to light the inside of the tank uniformly. The bottom of the tank was provided with 4~5 cm. of flowing water and the internal temperature was kept at 18~20°. Usually, 3~4 toads were placed in the tank as one group and submitted to light adaptation for 2.5 hrs. under observation.

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1) Part II : This Bulletin, **3**, 243(1955).